GRAVIDOMIMETIC PREVENTION OF BREAST CANCER

Thomas T. Andersen, H. I. Jacobson, J. A. Bennett, L. DeFreest, and D. McLeod

Albany Medical College

anderst@mail.amc.edu

From population studies, it is clear that pregnancy reduces the lifetime risk of breast cancer (BrCa). The major pregnancy-associated factor that contributes to this decrease in risk of BrCa is alpha-fetoprotein (AFP). We hypothesized that AFP acts in an endocrine manner to extinguish pre-malignant foci that would, later in life, develop into cancer. We have shown that AFP prevents the growth of human tumor xenografts growing in mice, that it is anti-estrogenic, and that it prevents the growth of estrogen-dependent cancers in cell culture. AFP itself is unsuitable as a potential drug for either the treatment or prevention of BrCa, not only because it is a large protein but also because it has been shown to have an immunosuppressive function. It has been suggested that this immuno-suppressive function leads to the early appearance of a small number of aggressive breast cancers shortly after pregnancy, but because of AFP's anti-estrotrophic activity, there is an overall decrease in tumor burden (in animal models) and tumor incidence (in women). We have identified and synthesized the active site of AFP (an octapeptide) and shown that it retains the full anti-oncotic activity of AFP. It prevents human BrCa growth in culture and as xenografts in immune deficient mice. It prevents the growth of tamoxifen-resistant MCF-7 human breast cancer xenografts and impedes the trophic effects of estrogen or tamoxifen on the uterus. Due to its small size, ease of synthesis, stability during storage, single (anti-oncotic) function, potency, and lack of apparent toxicity, this peptide may serve as a lead compound from which orally active agents for the treatment and/or prevention of BrCa could be generated. The peptide, in so far as it has ability to serve as a surrogate for intact AFP, should be gravidomimetic, and should prevent BrCa.

To test the hypothesis that the peptide can prevent BrCa, we utilized the N-Nitroso-N-methyl urea (NMU)-induced breast cancer model in rats. This model generates a high percentage of estrogen-receptor-positive breast cancers. We have initiated a dose-finding study in which four log doses of peptide were administered daily beginning 10 days after NMU treatment, and lasting for 23 days, a time period that mimics pregnancy. Treatment with peptide was then discontinued, and animals were palpated daily for 100 days. The number of animals with tumors, number of tumors per animal, time to generation of palpable tumors, and mass of tumors (at autopsy) were noted as endpoints, and weight, weight gain, cage activity and fur texture were used as gross assessments of toxicity. The study was not complete at the time of Abstract submission, but no evidence of toxicity due to peptide has yet been noted. Early generation of tumors associated with pregnancy was not observed, suggesting that the anti-oncotic site of AFP does not posses immunosuppressive activity. Pre-100 day data indicate fewer cancers in the Peptide- compared to No Peptide groups. We conclude that the model can appropriately assess prevention capability and should generate data concerning dosages for use in assessing the gravidomimetic potential of this AFP-derived peptide.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0472 supported this work.

RETINOIC ACID RECEPTOR AND RETINOID X RECEPTOR EXPRESSION IN BREAST CANCER AND THE RESPONSE OF CANCER CELLS TO RETINOIDS

X. Peng, L. Bratescu, J. Graves, R. R. Mehta, T. K. Das Gupta, and K. Christov

Department of Surgical Oncology, University of Illinois, Chicago, IL 60612

christov@uic.edu

Over the last 10 years, retinoids have been extensively studied for their potential chemopreventive and antitumor activity. Among them, *all trans*-retinoic acid (atRA), 9-*cis*-retinoic acid (9cRA), and 4-(hydroxyphenyl) retinamide (4-HPR) have shown promising inhibitory effects on mammary carcinogenesis and other carcinogenesis models. These retinoids are currently used in clinical trials for prevention and treatment of cancer. In this study, we evaluated the expression of retinoic acid receptors (RARs, α, β, and γ) and retinoid X receptors (RXRs, α, β, and γ) in 31 breast carcinomas and in early passages of breast cancer cells. We also assessed the response of tumor cells in vitro to the above retinoids. Polyclonal antibodies from Santa Cruz Biotech, Inc. (Santa Cruz, CA) were used for identification of RARs and RXRs expression in tumors by immunohistochemistry. Receptor expression was assessed at mRNA and protein levels by RT-PCR and Western blot. From 31 tumors examined, 12 were ER-negative. Most tumors expressed RARα (27 of 31), RARγ (30 of 31) and RXRα (30 of 31). RARα was predominantly expressed in the nucleus, although, in individual tumor cells, nuclear and cytoplasmic staining was also observed. RARβ was expressed in 19 of 31 tumors examined. In 6 tumors, cytoplasmic localization of RARβ was observed. RARγ and RXRα were localized in the nucleus or in both the nucleus and cytoplasm of tumor cells. RXRβ was found in the nucleus and/or cytoplasm of 21 of 31 tumors. In 7 tumors, the cytoplasmic location of RXRβ predominated. RARs and RXRs were also assessed in 9 primary cultures of breast cancer cells isolated from breast carcinomas in our department and grown *in vitro* (3-10 passages). RARα, RARγ, RXRα and RXRβ were variably expressed at the protein level in most of the tumor cells (7 of 9 tumor cell cultures). RARβ2 (50 kDa) was lacking in all 9 tumors. However, another protein band at 60 kDa was identified in 6 of 9 tumors by anti-RARβ. At the mRNA level, RARα, RARγ, RXRα were expressed in all tumors. But only in 1 tumor, RARβ was not detectable. When treated with atRA, 9cRA, or 4-HPR, 4 of 9 tumors were resistant to all retinoids, 3 tumors were most sensitive to atRA, and 2 tumors to 4-HPR. Taken together, our data indicate that breast carcinomas differ in the expression pattern of RARs and RXRs and that the early passages of breast cancer cells are differentially sensitive to retinoids. These data suggest that a preliminary *in vitro* testing of the response of breast tumor cells to various retinoids may help in selecting the most efficacious retinoid for breast cancer prevention and therapy trials.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9221 supported this work.

PROSPECTIVE STUDY OF ESTROGENS DURING PREGNANCY AND RISK OF BREAST CANCER

Pentii K. Siiteri, Robert I. Sholtz, Piera M. Cirillo, Richard D. Cohen, Roberta E. Christianson, Barbara J. van den Berg, William R. Hopper, and Barbara A. Cohn

Public Health Institute

psiiteri@aol.com and crwch@emf.net

Breast cancer prevention and control may benefit by elucidation of natural protective mechanisms. Pregnancy is an excellent model for studying protective mechanisms under conditions of promotion, when women are exposed to a variety of growth factors and hormones. The purpose of our research was to test the hypothesis that maternal serum levels of steroid hormones can explain novel protective associations that we recently reported for placental characteristics.

The study population is a subset of the Child Health and Development Studies, with more than 40 years of follow-up on 15,000 women. Women entered the study during pregnancy between 1959 and 1967,were members of Kaiser Foundation Health Plan and resided near Oakland, California. Extensive data were assembled through interviews, and examinations, including a standardized placental examination. Serum samples were stored, frozen at -20 degrees C. The design was a prospective case-cohort study with follow-up conducted via linkage to the California Cancer Registry. Cases are defined as invasive breast cancer diagnoses or deaths as of 1997. The first study pregnancy resulting in a live born, singleton birth was eligible. Pregnancy hormones were assayed for subjects with placental data (N=204 cases and 434 subcohort members). Pregnancy steroids were assayed following celite column chromatography for estrone and testosterone or by direct assays, which were validated by celite column chromatography, for estradiol and estriol. We verified the integrity of steroid assays in stored samples.

We found a protective association for the percent of estrogens present as estriol and this association had a significant linear trend ($p<0.01$). The protective association increased monotonically by quartile of estriol percent. Breast cancer risk was reduced by 58% for the 4^{th} quartile of estriol percent compared to the 1^{st} quartile of estriol percent (95% CI=26% reduction to 77% reduction). The estriol percent was higher in both Asian and Hispanic women, who are known to have reduced risk of breast cancer.

Our findings are consistent with an earlier hypothesis that estriol, an estrogen largely of fetal origin that rises 1,000-fold during pregnancy, protects against maternal breast cancer by antagonizing the effects of the active estrogen, estradiol.

If confirmed, these results could lead to breast cancer prevention or treatment regimens that seek to block estradiol action using estriol, similar to treatments based on the synthetic anti-estrogen, tamoxifen.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9358 supported this work.

THE ROLE OF CYCLOOXYGENASE IN BREAST CANCER

Namita Kundu, Miriam Smyth, Leigh Samsel, and Amy M. Fulton

Department of Pathology and Greenebaum Cancer Center, University of Maryland and Baltimore Veterans Affairs Medical Center

afulton@umaryland.edu

We have examined the role of Cyclooxygenase (Cox) expression in the behavior of murine mammary tumors that serve as a model of metastatic breast cancer. We have determined that expression and activity (prostaglandin synthesis) of the Cox-2 isoform are positively correlated with increased tumorigenic and metastatic potential. All mammary tumor cell lines expressed both Cox-1 and Cox-2 isoforms in vitro, but upon transplantation to syngeneic hosts, only metastatic lesions have detectable Cox-2. Thus, in the tumor milieu, Cox-2 expression can be regulated differently in metastatic vs nonmetastatic lesions. Administration of the dual Cox-1/Cox-2 inhibitor indomethacin to tumor-bearing mice results in significant inhibition of tumor growth and metastasis. Interestingly, although Cox-2 rather than Cox-1 is most closely correlated with tumor behavior, either Cox-1 or Cox-2 selective drugs also result in tumor inhibition. These studies suggest that Cox-1 as well as Cox-2 may be important therapeutic targets. We have begun to examine the mechanism by which these drugs control tumors. Treatment of tumor cells with either Cox-1 or Cox-2 inhibitors in vitro inhibits cell replication and increases the fraction of cells in G0/G1 of the cell cycle. This cell cycle arrest is associated with increased intracellular ceramide. The addition of exogenous cell-permeable C6 ceramide to cultured cells could mimic the effect of Cox inhibitors on cell cycle and growth inhibition. Unlike some other cells examined, these mammary tumor cells do not undergo apoptosis in response to Cox inhibitors. These preclinical studies suggest that Cox-2 and Cox-1 may be important new targets for the treatment of breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0322 supported this work.

TRANSFORMATION OF NORMAL MCF-12F BREAST EPITHELIAL CELLS AND SELECTIVE INHIBITION OF TRANSFORMED CELLS BY VITAMIN D ANALOG, 1-HYDROXY-VITAMIN D5

Erum A. Hussain, Rajeshwari R. Mehta, Tapas K. DasGupta, and Rajendra G. Mehta

Surgical Oncology, University of Illinois at Chicago

ehussa3@uic.edu

Several epidemiological studies had suggested a correlation between increased breast cancer mortality rates and lower serum levels of vitamin D3.The active form of vitamin D (1,25(OH) 2D3) has now been well recognized as an effective suppressing agent for leukemia, breast, colon, and prostate cancers. However, due to its hypercalcemic activity it is toxic at levels that are necessary for its chemopreventive effects. Therefore, much attention has been paid to developing non-toxic analogs of vitamin D. We have been studying an analog of vitamin D, 1-hydroxy-24-ethyl Cholecalciferol (VD5), for the past three years. This analog has shown antiproliferative and differentiation-inducing effects in carcinogen-transformed mouse mammary gland organ culture (MMOC) and breast cancer cells in vitro with little or no calcemic activity in vivo. VD5 inhibited preneoplastic lesions and growth of carcinogen treated MMOC, but it had no effect on the growth of normal MMOC. Consequently, we proposed to transform a normal breast epithelial cell line MCF12F and to study the mechanism of action of VD5 on the growth of normal versus transformed cell lines. MCF-12F cells were transformed with DMBA and MNU, and resulting cell lines were designated MCF-12FDMBA and MCF-12FMNU, respectively. To study the growth effects of VD5 on these cell lines, we performed cell count, MTT assay and FACS cell cycle analysis. Our results showed that the rate of growth of the transformed cells increased five fold and there was a loss of contact inhibition in the transformed cell lines. Cell count and MTT assay showed 40-70 % growth inhibition of MCF-12FDMBA and MCF-12FMNU with VD5 treatment, while no effect was observed on the normal cells. Likewise, VD5 treatment arrested the MCF-12FDMBA and MCF-12FMNU cells in G-1 phase of the cell cycle. While VD5 treated MCF-12F were not different from control. In conclusion, VD5 is effective in suppressing growth of carcinogen-transformed MMOC and MCF-12FDMBA and MCF-12FMNU cells, while it does not affect the growth or morphology of normal MMOC or normal breast epithelial cells. This suggests a selective effect of VD5 on cancer cells. Hence, VD5 can be developed as a promising chemopreventive agent.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0272 supported this work.

CONJUGATED LINOLEIC ACID MODULATION OF MAMMARY STROMAL DIFFERENTIATION CONTRIBUTES TO ITS CHEMOPREVENTIVE ACTIVITY IN MAMMARY CARCINOGENESIS

Margot M. Ip, Patricia A. Masso-Welch, Clement Ip, and Danilo Zangani

Department of Pharmacology and Therapeutics,
Roswell Park Cancer Institute, Elm and Carlton Streets
Buffalo, NY 14263

margot.ip@roswellpark.org

Conjugated linoleic acid (CLA) is a trace fatty acid found in meat and dairy products, which inhibits rat mammary carcinogenesis at low levels in the diet. In part, CLA exerts this effect by acting directly on the mammary epithelium. The objective of the current studies was to determine if CLA might also act indirectly, by modifying the mammary stroma. To examine this, we investigated the effect of CLA on a multipotent stromal cell population which is present in the rat mammary gland, and which is able to acquire a fibroblastic, adipocyte or endothelial phenotype, depending on culture conditions. In these experiments, t10,c12-CLA was found to be a potent adipogenic factor, stimulating MSC to the adipogenic differentiation pathway even in the absence of exogenous hormonal supplementation; c9,t11-CLA was less effective. This effect of CLA was accompanied by a rapid loss in the DNA-binding activity of the PPARg/RXRa heterodimeric transcription factor complex, suggesting that PPAR gamma may play a key role in initiating the recruitment of MSC into the adipogenic pathway. Significantly, concurrent with MSC differentiation along the adipogenic lineage, there was a decreased ability of MSC to form microcapillary networks in vitro on an EHS tumor-derived reconstituted basement membrane (RBM). This suggested that CLA might inhibit angiogenesis in vivo. To test this, mice were fed diets with or without CLA for 6 weeks, and then injected subcutaneously with an angiogenic gel substrate composed of RBM supplemented with bFGF and heparan sulfate. One week later, the RBM pellets were harvested and examined histologically. These studies demonstrated that functional angiogenesis (formation of red blood cell-containing vessels) was decreased by ~80%. CLA also significantly decreased serum and mammary gland concentrations of vascular endothelial growth factor (VEGF), and the mammary gland VEGF receptor, flk-1. In summary, the ability of CLA to modulate mammary stromal cell differentiation and decrease angiogenesis may contribute to its efficacy in inhibiting mammary carcinogenesis.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0286 supported this work.

ANTIPROLIFERATIVE EFFECTS OF INDOLE-3-CARBINOL-BASED COMPOUNDS ON HUMAN BREAST CANCER CELLS

Vaishali Kerekatte, Lucy Kong, Leonard F. Bjeldanes, and Gary Firestone

Department of Molecular and Cell Biology and
Department of Nutritional Sciences and Toxicology,
University of California at Berkeley

vkerek@uclink.berkeley.edu

One of the critical issues in controlling breast cancer is the need to develop therapeutics that can selectively target estrogen-dependent as well as estrogen-independent mammary tumors with reduced side effects. Indole-3-carbinol (I3C), a naturally occurring compound from vegetables of the Brassica family (cabbage, brussels sprouts, broccoli, kale), has been shown to be an effective chemopreventive agent against a variety of cancers including breast tumor models. Studies from our laboratory have shown that direct exposure to I3C activates a novel antiproliferative pathway independent of estrogen-receptor signaling and causes a G1 cell cycle arrest of both estrogen-responsive and nonresponsive breast cancer cells. Moreover, a combination of I3C and tamoxifen, an antiestrogen currently used in breast cancer therapies, inhibits the growth of estrogen-responsive breast cancer cells more stringently, compared to the effects of either compound alone. As a necessary first experimental step for the development of I3C-based therapeutic compounds, we have initiated studies on certain natural and synthetic I3C-based compounds to test their anti-proliferative effects on human breast cancer cells in culture and in vivo. The effects on cell cycle control were monitored by the incorporation of thymidine (S phase) and flow cytometry, as well as by the selective down-regulation of CDK6 expression, which is a hall mark response to I3C in MCF7 breast cancer cells. Several I3C-based compounds were found to be more potent than the I3C parent compound using these assays. As an in vitro measure of tumor formation, the indoles were tested alone, or in combination with tamoxifen, in a soft agar foci assay using estrogen responsive MCF7 cells. Each I3C-based indole that induced a G1 block in cell cycle progression were found to ablate cell foci formation and synergized with tamoxifen at non-optimal concentrations. Moreover, several other natural I3C derivatives were found to have no anti-proliferative effects in these assays. The in vivo effects of the indoles are being tested on MCF7-derived xenographs in athymic mice. Our studies suggest that indole compounds can be developed as potential chemotherapeutic agents for the treatment of breast cancer with reduced side effects.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0176 supported this work.

DIETARY FOLATE DEFICIENCY SUPPRESSES MAMMARY TUMORIGENESIS IN A CHEMICAL CARCINOGEN RAT MODEL OF BREAST CANCER

Joannae Kotsopoulos, Kyoung-Jin Sohn, Rochelle Martin, Richard Renlund, Colin McKerlie, Stephen Hwang, Alan Medline, and Young-In Kim

Departments of Laboratory Medicine and Pathobiology, Medicine and Nutritional Sciences, University of Toronto

youngin.kim@utoronto.ca

Epidemiological studies have suggested that dietary folate intake is inversely related to the risk of breast cancer. This study investigated the effect of dietary folate on the development and progression of N-methyl-N-nitrosourea (MNU)-induced mammary tumorigenesis in rats. Weanling, female Sprague-Dawley rats were randomized to receive an amino acid-defined diet containing either 0 mg (moderately folate deficient; n=22), 2 mg (basal dietary requirement [control]; n=20) or 8 mg (supplemented; n=20) folate/kg diet. At 50 days of age, all the rats received an intraperitoneal injection of MNU (50 mg/kg body weight) and the initial dietary interveniton was continued for additional 23 weeks. At necropsy, all macroscopic mammary tumors were identified and histologically confirmed for adenocarcinoma or its precursor, adenoma. Serum folate concentrations accurately reflected dietary folate levels at the time of MNU administration and at necropsy ($P<0.001$). The mean folate concentrations of the normal mammary gland of the folate-deficient group were significantly lower than those of the control and folate-supplemented groups ($P<0.001$), whereas no significant difference between the control and folate-supplemented groups was observed. The final incidence of mammary tumors in the folate-deficient group was significantly lower than that of the control and folate-supplemented groups (55% versus 90% and 75%, respectively, $P=0.04$). Kaplan-Meier analyses also demonstrated similar cumulative tumor incidence trends ($P=0.06$). By contrast, dietary folate supplementation did not significantly modulate both the final and cmulative incidences of mammary tumors compared with the control group. Dietary folate status had no significant effect on mean volume, weight, latency or multiplicity of mammary tumors. These data suggest that dietary folate deficiency of a moderate degree suppresses mammary tumorigenesis in this model. By contrast, dietary folate supplementation at 4x the basal dietary requirement does not significantly modulate mammary tumorigenesis. The role of folate in mammaray tumorigenesis needs to be clarified in future studies for safe and effective prevention of breast cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0428 supported this work.

CLINICAL TRIAL OF SOY ISOFLAVONES PRIOR TO BREAST CANCER SURGERY

Omer Kucuk, Fazlul Sarkar, Lisa Newman, David Bouwman, Volkan Adsay, Zora Djuric, Daniel Doerge, Ralph Parchment, Mousumi Banerjee, Adhip Majumdar, Lynn Hryniuk, and William Hryniuk

Karmanos Cancer Institute, Wayne State University,
3990 John R Street, Detroit, MI 48201

kucuko@karmanos.org

Our studies investigate the in vivo effects of soy isoflavones on human breast tissues obtained from lumpectomy/mastectomy specimens. We investigate the effect of increased tissue concentration of isoflavones for a period of three weeks on breast cell proliferation, differentiation and cell cycle regulatory proteins. Patients with ductal carcinoma in situ (DCIS) or invasive breast cancer are randomly assigned to take 100 mg soy isoflavone (NovasoyTM, Archer Daniels Midland Company, Decatur, Illinois) or placebo daily for three weeks prior to surgery. Plasma isoflavone levels are measured at baseline and after three weeks in both groups. Tissue isoflavone levels are measured on samples from benign breast tissues in both groups. Biomarker studies are performed on surgical specimens by immunohistochemistry and Western blot. These studies will enable us to determine if a short duration of exposure to increased tissue levels of isoflavones will modulate biomarkers of cell differentiation (Cx43), adhesion (E-cadherin), proliferation (MIB-1), and cell growth and apoptosis (bcl-2, bax, p21, EGFR, cyclin D1, CDK5, CDK6) in benign, pre-malignant and malignant areas of breast epithelial tissues. We will also measure plasma equol, a metabolite of soy isoflavones, and lipid peroxidation products. DNA oxidation products will be measured on peripheral blood lymphocytes. Metabolites of 5-lipoxygenase (5-HETE, LTB4) and cycloxygenase (PEG2) pathways will also measured in the plasma. We will also assess ERRP (EGFR Related Protein) levels in tissues by immunohistochemistry and by Western blot. Biomarker studies on the patients randomized on this study will be presented.

We have conducted a pilot study in six women, who took soy isoflavones 50 mg (NovasoyTM) daily for three weeks. DNA was isolated from the nuclei of peripheral blood lymphocytes and analyzed for levels of 5-hydroxy-methyl-2'-deoxyuridine (5-OHmdU) by gas chromatography-mass spectrometry. The mean level of 5-OHmdU was decreased by 35% (relative to baseline) after 1 week and by about 50% after 2 weeks and 3 weeks of supplementation. Mean plasma levels of 8-isoprostane also decreased after supplementation.

Original work supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8353.

GENISTEIN PROGRAMMING AGAINST BREAST CANCER

Coral A. Lamartiniere, Ph.D., and Jun Wang, M.D.

University of Alabama at Birmingham,
Birmingham, AL 35294

coral.lamartiniere@ccc.uab.edu

Asian women, consuming a diet high in soy, have a low incidence of breast cancer. We have hypothesized that one component of soy, genistein, protects against breast cancer by programming the mammary gland to be less susceptible for this cancer. For mammary cancer studies, Sprague-Dawley rats were exposed to genistein via the diet (250 mg genistein/kg AIN-76A diet). Genistein exposure was 1) prenatally, 2) prepubertally (days 1-21), and 3) adult (days 100-230), and 4) prepubertally and adult. Dimethylbenz[a]anthracene (80 mg/kg body weight) was administered by gavage at day 50 postpartum to induce mammary tumors. Mammary cancer chemoprevention was demonstrated after prepubertal, and combined prepubertal and adult genistein treatments but not after prenatal- or adult-only treatments, demonstrating that timing of exposure to genistein is important for mammary cancer chemoprevention. Mechanistically, the initial effect of prepubertal exposure to genistein was to up-regulate estrogen receptor signaling (increased progesterone receptor) and EGF-receptor expression. This enhanced mammary gland maturation as measured in mammary whole mounts (conversion of terminal end buds to lobules). Beta-casein expression was elevated in mammary glands of genistein treated rats, indicating enhance cell differentiation. This was followed by terminal end buds of adult female rats having reduced EGF-receptor expression.

Exposure Period	Mammary Tumor Multiplicity
No Genistein	8.9
Prenatal Genistein	8.8
Adult Genistein (after tumors)	8.2
Prepubertal Genistein	4.3
Prepubertal and Adult Genistein	2.8

We conclude that prepubertal genistein exposure enhances mammary gland differentiation to alter the molecular blueprint, regulating specific sex steroid receptors and growth factor signaling pathways. The most sensitive period for mammary cancer chemoprevention in the rat is the prepubertal period and in the human is probably the adolescent period. Our laboratory data are consistent with the epidemiological report showing an inverse relationship between adolescent soyfood intake and breast cancer incidence later in life.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0118 supported this work.

CELECOXIB INHIBITS THE GROWTH OF ESTABLISHED MAMMARY TUMORS IN HER2/NEU MICE

Susan Lanza-Jacoby, Sheldon Miller, and Harjeet Sembi

Department of Surgery, Jefferson Medical College,
Philadelphia, PA 19107

susan.lanza-jacoby@mail.tju.edu

There is abundant evidence in support of the role of cyclooxygenase (COX)-2 inhibitors in the prevention of human cancers. Recent studies indicate that the COX-2 inhibitor, celecoxib was able to prevent the development of DMBA-induced mammary tumors and also to shrink established tumors in rats. In this study, we evaluated whether the celecoxib would protect against mammary carcinogenesis in the HER2/neu mouse model.

In the first study female, 4-week old HER2/neu mice were assigned to one of two groups. The control group (n = 26) was fed a standard rodent diet #5029 diet (Purina) and the celecoxib group (n = 26) was fed the #5020 diet supplemented with 1500 mg/kg celecoxib (Pharmacia). Mice were monitored weekly for palpable tumors; tumor diameters were measured every two weeks. The mice were sacrificed when the tumors were 20 mm in diameter or when the mice were 14 months old. COX-2 protein in mammary tissue and tumors was determined by Western analysis. In a second study, HER2/neu mice were assigned to a control group (n = 8) or a celecoxib group (n = 8) when the mice were six months old and had a palpable mammary tumor of < 0.05mm. Tumor diameters were measured weekly for 8 weeks.

Feeding celecoxib had no significant effect on tumor latency (time of appearance of first tumor) or tumor multiplicity (number of tumors /mouse). Celecoxib produced a small but not significant effect on tumor incidence (control group = 83% and celecoxib group = 73%). COX-1 and COX-2 proteins were present in normal mammary tissue and mammary tumors. Celecoxib treatment did not alter the expression of COX-2 in the mammary tissue. However, celecoxib treatment significantly decreased the growth of established mammary tumors. There was a 212% increase in tumor diameter in the mice fed the #5020 diet over the 8-week treatment period compared with 25% increase in the mice fed the #5020 diet with celecoxib ($p<0.05$).

These results indicate that celecoxib is not effective in preventing mammary tumors in transgenic mice that overexpress HER2/neu. However, celecoxib does retard the growth of established tumors and thus, may have a role in treating HER2 positive breast tumors.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0299 supported this work.

DIINDOLYLMETHANE (DIM) AND RELATED COMPOUNDS AS ANTICANCER AGENTS

Jeong-Eun Lee, Andrew McDougal, Kavita Ramamoorthy, Mona Sethi-Gupta, Derek Morrow, Jessica Stewart, and Stephen Safe

Department of Veterinary Physiology and Pharmacology,
Texas A&M University, College Station, TX 77843-4466

ssafe@cvm.tamu.edu

Indole-3-carbinol (I3C) conjugates are highly expressed in cruciferous vegetables such as broccoli, Brussels sprouts and cauliflower, and I3C and related phytochemicals have been linked to the anticarcinogenic activity of cruciferous vegetables and/or their extracts in breast and other cancer cell models. I3C is highly acid labile and forms multiple condensation products including diindolylmethane (DIM) which is relatively stable in acid and exhibits many activities similar to I3C. DIM has been used in this study as a model for developing new DIM-based drugs for treatment of breast cancer. DIM inhibits 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor growth in female Sprague-Dawley rats and in athymic nude mice bearing MCF-7 breast cancer cell xenografts and an implanted estrogen pellet. In addition, we have also investigated a series of ring-substituted alkyl- and halogen-substituted DIM analogs in breast cancer cells (T47D/MCF-7) and in the DMBA-induced rat mammary tumor models. The results showed that the following compounds significantly inhibited estrogen-dependent cell proliferation and rat mammary tumor growth at doses ≤ 500 μg/kg/day; 4,4'-dichloro-, 6,6'-dichloro-, 5,5'-dibromo-, 1,1'-dimethyl-, 2,2'-dimethyl-, 5,5'-dimethyl- and 1,1',2,2'-tetramethylDIM.

Subsequent studies also show that ring-substituted DIMs weakly transform the cytosolic aryl hydrocarbon receptor (AhR) complex as determined in gel mobility shift assays and also induce reporter gene activity in breast cancer cells transfected with a Ah-responsive construct containing a dioxin responsive element. The results of Ah-responsiveness assays suggest that the ring-substituted DIMs are weak Ah receptor agonist, and this is inconsistent with their potent antitumorigenic activity and inhibition of estrogen receptor-positive and -negative breast cancer cell growth. Current studies are focused on other mechanisms that contribute to the anticarcinogenic activities of ring-substituted DIMs.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9396 supported this work.

EVALUATION OF GOSSYPOL-CONTAINING COTTONSEED OIL AS A DIETARY CHEMOPREVENTIVE AGENT FOR ESTROGEN-INDUCED MAMMARY CARCINOGENESIS

Young C. Lin, Suling Liu, Samuel Kulp, Yasuro Sugimoto, Jiahua Jiang, Hsiang-Lin Chang, Michael K. Dowd, and Peter Wan

Laboratory of Reproductive and Molecular Endocrinology,
College of Veterinary Medicine, Ohio State University
Comprehensive Cancer Center, The Ohio State University,
Columbus, OH

lin.15@osu.edu

Gossypol (GP) is a polyphenolic pigment present in cottonseed products that are consumed by humans and animals. Recently, the anticancer activities of GP have become a focus of research in our lab and others. We have reported that GP induces antiproliferative effects on cancerous human breast cells and human breast cancer (hBC) cell lines, and that GP can prevent estrogen-induced tumorigenesis in the DES-induced renal adenocarcinoma hamster model. We hypothesize that GP-induced antiproliferative effects on cancer cells are mediated by the enhanced expression and production of TGF-ß. Our preliminary results show that GP-containing cottonseed oil (GP-CSO) exhibits potent antiproliferative activity against PC-3 cells. The effective antiproliferative dose range for GP-CSO is 524 - 2083-fold lower than that of chemically prepared GP and approximately 361,500 - 454,240-fold lower than the FDA-mandated limit for GP content in human foods (450 ppm). These in vitro findings indicate that GP in its natural form in CSO is apparently more potent than the pharmaceutical form of GP and that the consumption of cottonseed oil-containing foods may deliver sufficient quantities of GP to achieve desired biological effects.

Natural GP is a racemic mixture of 2 enantiomers, (+)GP and (-)GP. Results showed that, while (+)GP failed to effect 3H-thymidine uptake in either cell type, both (±)GP and (-)GP inhibited proliferation of both breast cancer epithelial cells (EC) and stromal cells (SC). (±)GP caused reductions of 15, 46 and 82% at 2.5, 5.0 and 7.5 µM, respectively, in EC, and reductions of 17, 28, 39 and 56% at 2.0, 3.0, 4.0 and 5.0 µM, respectively, in SC. (-)GP caused reductions of 33, 89 and 98% at 2.5, 5.0 and 7.5 µM, respectively, in EC, and reductions of 29, 51, 64 and 72% at 2.0, 3.0, 4.0 and 5.0 µM, respectively, in SC. RT-PCR results revealed that these reductions in proliferation were associated with decreased cyclinD1 and elevated TGFß mRNA levels. These results reveal that (-)GP is the major and more potent inhibitory component of (±)GP in human breast cancer cells and that this inhibition involves alterations of the cell cycle regulator cyclinD1 and the cell proliferation inhibitor TGFß.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0391 supported this work.

SELENIUM AND CANCER ANGIOPREVENTION: METABOLITE-SPECIFIC ANTIANGIOGENIC ATTRIBUTES

Junxuan Lu, Ph.D.,[1] Cheng Jiang, M.D.,[1]
Zaisen Wang, Ph.D.,[1] and Howard Ganther, Ph.D.[2]

[1]AMC Cancer Research Center, Denver, CO 80214; and
[2]University of Wisconsin, Madison, WI

luj@amc.org

The trace element nutrient selenium (Se) has been implicated as a potent chemopreventive agent for some cancers. Two large human clinical trials are ongoing to establish its efficacy for prostate and lung cancer prevention. The mechanisms of action remain largely unknown. Since angiogenesis is obligatory for solid tumor growth and progression, we investigated the anti-angiogenic attributes of two pools of Se metabolites, namely methylselenol or related monomethyl Se vs. hydrogen selenide, to define potential target proteins and cellular processes crucial to tumor angiogenesis inhibition. Data obtained with cell culture models showed that monomethyl Se potently inhibited endothelial expression of matrix metalloproteinase-2 (MMP-2) and cancer epithelial expression of vascular endothelial growth factor (VEGF). In contrast, Se forms that enter the hydrogen selenide pool lacked any inhibitory effect. The methyl Se-specific inhibitory effects on these proteins were rapid and primary actions, independent of inhibitory effects on mitogenic signaling at the level of MAPK1/2 and of cell apoptosis.

In addition, methyl Se exposure induced vascular endothelial G_1 arrest at physiologically relevant concentration ranges and induced caspase-mediated apoptosis at pharmacological dosages. We examined the hypothesis that methyl Se inhibits G_1 cycle progression by targeting specific protein kinase signaling pathway(s). We developed a synchronized human umbilical vein endothelial cell (HUVEC) G_1 progression model to evaluate the G_1-stage specific action of methyl Se. During stimulation by the bovine pituitary extract endothelial cell growth supplement (ECGS), methyl Se targeted a mechanism(s) in mid- to late-G_1. The PI3K inhibitors, wortmannin and LY294002, showed the same stage-specific inhibitory action on G_1 progression to S, while having no inhibitory effect on DNA synthesis once S phase had initiated. In contrast, the mitogen-activated protein kinase kinase (MEK) pathway appeared to be involved in signaling G_0/G_1 entry because the MEK inhibitor PD98059 moderately inhibited DNA synthesis when given before ECGS-stimulation, but did not have inhibitory effect after cell cycle had progressed for 6 h or beyond. Furthermore, MSeA exerted an additive inhibitory effect with wortmannin, but did not increase the inhibitory action of PD98059. Taken together, the results support a potent inhibitory activity of methyl Se on ECGS-stimulated vascular cell mitogenesis and the target(s) of this inhibitory activity appeared to be related to PI3K pathway.

Based on the low concentration of Se required for the methyl Se-specific inhibition of angiogenic cytokine VEGF expression in cancer cells and vascular endothelial expression of MMP-2 and anti-mitogenic action, we speculate that these activities may dampen angiogenic switching in early lesions *in vivo* to achieve cancer chemoprevention (i.e., angioprevention). The efficacy for an organ to enrich monomethyl Se metabolites and the nature of the angiogenic switch mechanisms in that organ site may contribute to the organ-specific cancer preventive efficacy of Se.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9061 supported this work.

CELECOXIB IN WOMEN AT INCREASED BREAST CANCER RISK

Iacono R, Flynn JT, and Sauter ER

Thomas Jefferson University

edward.sauter@mail.tju.edu

Background: Cyclooxygenase (COX)-1 and COX-2, are present in breast tumors and catalzye the conversion of arachadonic acid to prostaglandins. Prostaglandin E2 (PGE2) has tumor and cell growth promoting activity, and is overexpressed in human breast cancer.

Objective/hypothesis: We propose a pilot study of celecoxib administered to women at increased breast cancer risk, defined as a projected 5 year Gail model risk of invasive breast cancer > 1.66%, as well as women with a history of ductal carcinoma in situ or invasive breast cancer. Our hypothesis is that celecoxib will be concentrated in breast fluid compared to corresponding plasma, and that it will decrease PGE2 levels in nipple aspirate fluid (NAF) and in plasma.

Specific Aims: To determine if 1) celecoxib is delivered to the breast, and 2) PGE2 levels in NAF and plasma increase after a 2 week course of celecoxib, then return to baseline 2 weeks after stopping the medication.

Study Design: Both pre- and postmenopausal women will undergo nipple aspiration and blood draw three times: before, 2 weeks after starting celecoxib, and 2 weeks after stopping the medication. Each woman serves as her own control.

Results: Thus far we have enrolled 4 women on trial. NAF has been collected successfully in all subjects at each time point. PGE2 levels were measurable in all NAF samples, and were significantly higher in NAF than in matched plasma.

Future plans: Our hope is to determine if PGE2 levels significantly decrease after celecoxib administration, and if they return to baseline 2 weeks after discontinuing medication.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0427 supported this work.

SE-METHYLSELENOCYSTEINE ACTIVATES CASPASE-3 IN MOUSE MAMMARY CANCER

Raghu Sinha, Emmanual Unni, and Uma Singh

Baylor College of Medicine, Houston, TX 77030

rsinha@bcm.tmc.edu

Se-methylselenocysteine [MSC] is a promising chemopreventive agent against mouse mammary cancer and its mechanism of action in not well understood. The overall goal of this study was to examine the primary and secondary interactions between MSC and caspases in three model systems: a cell free system, an in vitro model for mouse mammary epithelial tumor cells and an animal model for TM6 hyperplasia.

Cell free system was designed by the addition of cytosolic fraction from TM6 cells, dATP and cytochrome c and the activation of caspase(s) was examined after incubation with MSC. For the in vitro model, TM6 mouse mammary epithelial tumor cells were synchronized following serum and growth factor deprivation. MSC-induced caspase enzyme activity, DNA fragmentation assay and poly(ADP-ribose) polymerase [PARP] cleavage were monitored in a time-dependent manner. TM6 hyperplastic outgrowth [TM6-HOG] model was used to compare animals fed 0.1ppm MSC and 3.0ppm MSC in diet.

MSC did not activate caspases in the cell free system. In vitro, 50uM MSC inhibited TM6 growth and induced apoptosis at 48hr. Caspase-3 was activated either by a 30-minute treatment or a continuous exposure to 50uM MSC followed by PARP cleavage and DNA fragmentation. 3.0ppm MSC in diet for 10 weeks decreased growth in mammary fat pads and caused a 5-fold increase in caspase-3 activity as compared to 0.1ppm MSC fed mice. At the end of 22 weeks, 77% of animals in 3.0ppm MSC group had tumors as compared to 100% tumors in 0.1ppm MSC fed animals. The size of TM6 tumors in the former group was smaller. A cDNA array analysis of tumors from these groups showed that osteopontin [OPN] gene was down regulated following 3.0ppm MSC treatment. These data were supported by reduction of OPN gene expression and an inhibition of TM6 migration following 24hr treatment with 100uM MSC in a transwell setup in vitro.

We conclude that MSC mediates PARP cleavage and apoptosis by activating one or more caspases in synchronized TM6 cells and the events are dependent on the duration of treatment. A dose of 3.0ppm MSC activated caspase-3 in mammary fat pads following 10 weeks of treatment when compared to mice fed 0.1ppm MSC. OPN and caspase-3 are potential surrogate markers for MSC-inhibition of mammary cancer.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9076 supported this work.

ROLE OF SERMS AND REXINOIDS IN PREVENTION AND TREATMENT OF BREAST CANCER IN RATS

Michael B. Sporn, Nanjoo Suh, Mara H. Rendi, Charlotte R. Williams, and Renee Risingsong

Department of Pharmacology, Dartmouth Medical School

michael.b.sporn@dartmouth.edu

We have been studying the use of combinations of preventive and therapeutic agents in a rat breast cancer model that uses nitrosomethylurea as the carcinogen. We have previously shown that the selective estrogen receptor modulator (SERM), arzoxifene, is a potent agent for prevention of breast cancer in this animal model (Suh, N., Glasebrook, A., Palkowitz, A., Bryant, H., Burris, L., Starling, J., Pearce, H., Williams, C., Peer, C., Wang, Y., Sporn, M. B. Arzoxifene, a new selective estrogen receptor modulator for chemoprevention of experimental breast cancer. Cancer Res., 61: 8412-8415, 2001). Other studies have shown that ligands that are selective for binding to the nuclear receptors known as RXRs ("rexinoids") are also effective agents for prevention of breast cancer in this model (see for example, Bischoff, E. D., Gottardis, M. M., Moon, T. E., Heyman, R. A. & Lamph, W. W. Beyond tamoxifen: the retinoid X receptor-selective ligand LGD1069 (TARGRETIN) causes complete regression of mammary carcinoma. Cancer Res. 58: 479-484, 1998). We now report that arzoxifene and the rexinoid LG 100268 are strongly synergistic for both prevention and treatment of estrogen receptor-positive breast cancer in this animal model. Mechanistic studies in cell culture suggest that enhancement of stromal-epithelial interactions, mediated by the cytokine, TGF-beta, may contribute to the synergistic effects that we have observed. The possible clinical use of the combination of arzoxifene and LG 100268 either for prevention of breast cancer in women at high risk, or for treatment of women in the adjuvant setting, or for treatment of end-stage disease, should now be considered. We thank Andrew L. Glasebrook, Timothy A. Grese, and Alan D. Palkowitz (Lilly Research Laboratories) and William W. Lamph and Richard A. Heyman (Ligand Pharmaceuticals) for their contributions to this work.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9168 supported this work.

INHIBITION OF DIETHYLSTILBESTROL-INDUCED MITOCHONDRIAL DNA ADDUCTS BY DIALLYL SULFIDE

Ronald D. Thomas, Ph.D.

Environmental Toxicology Program, College of Pharmacy,
Florida A&M University, Tallahassee, FL 32307

ronald.thomas@famu.edu

Among women ages 30-60**,** breast cancer is the second leading cause of death. Estrogen metabolism has been shown to play a significant role in Breast cancer formation. Diallyl sulfide (DAS), a component of garlic, has been shown to prevent cancer in animals. The implicated mechanism of chemoprevention by DAS is via metabolic inhibition.

The study of estrogen-related DNA damage has been focused on nuclear (n)DNA. However, mitochondrial (mt) DNA is the primary site of attack by reactive chemicals. Mitochondrial alterations such as, gene mutatuons, gene expression, and the amount and forms of mtDNA have been associated with cancer. Previously, we have demonstrated that microsomes, mitochondria, and nuclei isolated from breast tissue of female ACI rats catalyze redox-cycling of DES producing reactive metabolites, one of which being DES quinone. DAS inhibits the formation of this quinone. We propose that mitochondrial DNA alterations generated by DES may play a role in estrogen induced cancer and DAS will inhibit this damage therefore inhibiting breast cancer.

We hypothesize that DES is metabolized by the mitochondria to DES quinone which bind to mtDNA producing DNA adducts. We further propose that diallyl sulfide will inhibit the production of mtDNA adducts. To test this hypothesis we incubated mitochondria with 200ug DNA, 100mM DES, and/or 100mM DAS in an *in vitro* system. The DNA was extracted and analyzed by ^{32}P post-labeling. We also dosed female ACI rats with DES (150mg/kg) and DAS (200mg/kg). The DNA from breast tissue was isolated by enzyme digestion and solvent extraction. The DNA was then analyzed by ^{32}P post-labeling for adduct formation.

The *in vitro* study revealed that DES treatment produced DNA adducts in the mitochondrial activation system and DAS inhibited DNA adduct formation. The relative adduct level for the DES reaction was 61.5×10^{-7} and the relative adduct level for the DES/DAS reaction was 7.12×10^{-7}. Thus DAS inhibited DNA adduct formation by 89%. In the DNA isolated from rats treated only with DES an adduct level of 8.9×10^{-8} was produced and no adducts were produced in the rats dosed with DAS and DES.

We have demonstrated that DAS inhibits DNA adduct formation in the mitochondria *in vitro* and *in vivo*. Therefore, a possible mechanism of DAS inhibition of breast cancer is via the inhibition of mitochondrial estrogen metabolism.

Original work supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8081.

THE FATTY ACID-BINDING PROTEIN MAMMARY-DERIVED GROWTH INHIBITOR-RELATED GENE INDUCES MAMMARY GLAND DIFFERENTIATION IN TRANSGENIC MICE

Mingsheng Wang, Yiliang Liu, and Eric Shi

Long Island Jewish Medical Center,
New Hyde Park, NY 11040

wang@lij.edu

We have previously identified and characterized a novel tumor growth inhibitor and a fatty acid binding protein in human mammary gland and named it as Mammary derived growth inhibitor Related Gene MRG. MRG induces differentiation of mammary epithelial cells in vitro and its expression is associated with human mammary gland differentiation. To further define the role of MRG on mammary gland differentiation, a MRG transgenic mice model under the control of MMTV promoter was established and investigated. Expression of endogenous mouse MRG gene was significantly increased from the non-differentiated gland from control virgin mice to the differentiated gland from pregnant control mice. While there was limited lobulo-alveolar structure in control virgin mice, expression of MRG transgene in the mammary gland resulted in a significant increase in the formation of lobulo-alveolar structure. Consistent with the morphological change, expression of MRG also increased milk protein b-casein expression in the mammary gland. To study the mechanism of MRG-induced mammary gland differentiation, we investigated the Stat5 activation in the glands from the transgenic mouse vs. virgin control mouse. While activated Stat5 was not detectable in the non-differentiated control virgin gland, a significant Stat5 phosphorylation was observed in the differentiated virgin transgenic gland. Our data indicate that MRG is mediator of the differentiating effects of pregnancy on breast epithelium and overexpression of MRG in young nulliparous mice can induce differentiation.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0353 supported this work.

MODELING MULTIPLE TIME-TO-EVENT DATA USING SPLINES

Berhane, K., and Weissfeld, L. A.

Department of Preventive Medicine, University of Southern California and Department of Biostatistics, University of Pittsburgh

lweis@pitt.edu

Pseudosmoothers have been applied to time-to-event data, providing an extension of the proportional hazards model for a single outcome (Gray, 1994). We use this technique to extend the marginal models of Wei, Lin and Weissfeld (1989). This allows for greater flexibility in modeling the margins and makes formal development of inferential procedures possible. Applications to data from the NSABP-BCPT on the effectiveness of the drug Tamoxifen as a prevention tool against breast cancer will be discussed in detail. Results from extensive simulation studies on the small sample properties of the asymptotic tests will also be presented.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9356 supported this work.

MECHANISMS OF RETINOID ACTION IN BREAST CANCER CELLS

Bingzhen Lin, Hui Li, Siva Kumar Kolluri, and Xiao-kun Zhang

The Burnham Institute, Cancer Center,
10901 North Torrey Pines Road, La Jolla, CA 92037

blin@burnham.org

Vitamin A and its natural and synthetic derivatives, Retinoids, exert profound effects on cell proliferation, differentiations, and apoptosis, and are considered promising agents for prevention and treatment of breast cancer. The effects of retinoids are mainly mediated by two classes of nuclear receptor, the retinoic acid receptor (RARs) and the retinoid X receptors (RXRs). We previously found that the conventional retinoids, such as trans-RA, can promote apoptosis in breast cancer cell lines and that induction of apoptosis and growth inhibition by trans-RA is largely mediated by RAR beta. Induction of RAR beta in hormone-dependent breast cancer cells is mediated by RAR/RXR heterodimer that binds to the RA response element (beta RARE) in RAR beta promoter. However, RAR beta induction is lost in hormone-independent breast cancer cells. Here, we provide evidence that lack of orphan receptor COUP-TF is responsible for loss of RAR beta in hormone-independent breast cancer cells. We showed that expression of COUP-TF is required for trans-RA to induce RAR beta expression, growth inhibition, and apoptosis. Regulation of RAR beta expression by COUP-TF requires both a DR-8 element that binds strongly with COUP-TF and the beta RARE in the RAR beta promoter. In addition, we found that a class of RXR- selective retinoids could effectively induce RAR beta expression and growth inhibition in hormone-independent breast cancer cells. Our results demonstrated that the RXR-selective retinoids act through heterodimers formed by RXR and orphan receptor TR3, which bind to the beta RARE. Recent studies revealed that the retinoid 6-[3-(1-adamantyl)-4-hydroxy phenyl]-2-naphthalene carboxylic acid (AHPN/CD437) can effectively induce apoptosis of hormone–independent breast cancer cells. In studying its molecular mechanism of action, we found that TR3 expression but not its transactivation function mediates apoptotic effect of AHPN. Moreover, TR3 was exported from the nucleus to the cytoplasm, where it is targeted to mitochondria. This finding demonstrated a new paradigm in causing breast cancer cell apoptosis and suggest that TR3 is a promising molecular target for developing effective anti-breast cancer retinoids.

The U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0172 supported this work.

IDENTIFICATION AND CHARACTERIZATION OF TWO PHYTOESTROGEN-SPECIFIC GENES

Lakshmi Ramanathan and Wesley G. Gray

Department of Chemistry,
Southern University-Baton Rouge, LA

wggray@subr.edu

Phytoestrogens (PEs) are a group of compound presence in human diet that displays estrogenic-like properties. Several studies has demonstrated that populations that consumed large quantities of PEs has a reduce risk of estrogen dependent cancers. Although it has been shown that certain PEs modulates estrogen action, their biological role in cancer reduction remains unclear. Thus, the objective of this study was to identify a set of markers that could be used to assess PE function independent of their estrogenic properties.

Through the use of differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) and representational difference analysis of cDNA (cDNA-RDA), we have identified several PEs responsive genes from the human breast cancer cell, MCF-7 cells in response to PEs. Two of these biomarkers, PE13.2 and pNIC-D, have been characterized in greater detail.

The results presented here demonstrate the differential expression of two novel genes that specifically coding RNA transcript, previously not known to be regulated by neither PEs nor estradiol. Northern blot and Ribonuclease protection assays confirmed that the PE-13.2 transcript was up regulated by PEs (GE, 5- fold, ZE, 4- fold, RE, 6 -fold) but is non-responsive to estradiol. PE and estradiol, on the other hand, down regulated the pNIC-D transcript. The expression of these transcript were PEs specify and estrogen receptor dependent in that the anti-estrogen ICI inhibits the expression of PE-13.2 and reverse the inhibition of pNIC-D expression induces by the PEs and estradiol.

This study identifies two biomarkers that could be used to assess PEs functional and mechanistic action with out the influence of their estrogenic activity. Furthermore, this report demonstrates the present of PEs responsive genes that may be used to decipher the role of dietary estrogen at the molecular level.

The U.S. Army Medical Research and Materiel Command under DAMD17-00-1-0436 supported this work.

THE FATTY ACID-BINDING PROTEIN MRG INDUCES MAMMARY DIFFERENTIATION IN TRANSGENIC MICE

Mingsheng Wang, Yiliang E. Liu, Mahmut Y. Celiker, and Y. Eric Shi

Long Island Jewish Medical Center,
Albert Einstein College of Medicine

shi@lij.edu

There is an increasing public interest in chemoprevention by natural agents such as n-3 polyunsaturated fatty acids (PUFAs) DHA and EPA and the pregnancy-induced differentiation against breast cancer incidence. Little is known about the regional and developmental expression of locally acting factors in the mammary epithelium that interact with n-3 PUFA and exert differentiating effect during pregnancy. Within this content, a novel mammary derived growth inhibitor and a fatty acid binding protein (FABP) has been identified, characterized, and named Mammary derived growth inhibitor Related Gene (MRG). As a new member in the family of FABP, MRG has the highest binding affinity to n-3 PUFA DHA. MRG overexpression suppresses breast cancer cell growth in vitro and tumorigenesis in vivo. MRG induces differentiation of mammary epithelial cells and its expression is associated with human mammary gland differentiation. To further define the role of MRG on mammary gland differentiation, a MRG transgenic mice model under the control of MMTV promoter was established and investigated. While there is limited lobulo-alveolar structure in control virgin mice, a significant increase in the formation of lobulo-alveolar structure was observed in the gland from MMTV/MRG mice. Although, the magnitude of MRG effect is less than that of human placental hormone chorionic gonadotropin (hCG) stimulated formation of alveoli, the MRG-induced formation of alveoli is compatible to that of hCG and is significant vs. the control virgin mice. Overexpression of MRG also, as expected, increased milk protein beta-casein expression in the mammary gland. To study the mechanism of MRG-induced mammary gland differentiation, we investigated the Stat5 activation in the glands from the transgenic mouse vs. virgin control mouse. While activated Stat5 was not detectable in the non-differentiated control gland, a significant Stat5 phosphorylation was observed in the differentiated transgenic gland. Our data indicate that MRG is mediator of the differentiating effects of pregnancy on breast epithelium and overexpression of MRG in young nulliparous mice can induce differentiation.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9255 supported this work.